REMARKS:

Claim 21 is amended. Claim 33 is canceled without prejudice. Claims 1-32 and 34-72 are pending in the application. Reexamination and reconsideration of the application, as amended, are respectfully requested.

Claim 33 is objected to under 35 U.S.C. § 112 as being a duplicate of claim 32. In response, applicant cancelled claim 33. Thus, the objection is believed to be overcome.

Claim 21 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In particular, the Examiner states that claim 21 lacks an antecedent basis for the limitation "said target nucleic acid." In response, applicant amended claim 21 by replacing the term "target nucleic acid" with the term "target biopolymer." Claim 21 depends from claim 1. Claim 1 provides a sufficient antecedent basis for the term "target biopolymer" in claim 21. Thus, amended claim 21 is believed to comply with the requirements of 35 U.S.C. § 112, second paragraph.

Claims 1, 3, 7, 9, 11-14, 17-18, 22-28, 53, 55-58, 62, and 66-71 are rejected under 35 U.S.C. § 102(b) as being anticipated by Jahn et al., *Proceedings of the National Academy of Sciences, USA*, (1984), Vol. 81, pages 1684-1687 ("Jahn"). This rejection is respectfully traversed.

The method of this invention provides a novel method for the multiplex analysis of a sample using a microarray format that allows for the simultaneous interrogation of a single sample with multiple probes (page 7, lines 7-9). The method, according to the present invention, follows the Southern blot format by immobilizing a single sample containing one or more biopolymers of interest as discrete aliquots or printed elements on a substrate in the form of a microarray. According to the present invention, each printed element on the sample microarray contains an equivalent amount of the target biopolymer(s) of interest. This sample

microarray is then interrogated with one or more known probes (page 7, lines 12-19).

The instant specification defines the terms "microarray" and "sample microarray," as a collection of aliquots (S_1, S_2, S_3, etc. . . .) from a single sample (S) arranged on a substrate in a spatially defined and addressable manner. The dispensed aliquots are equivalent in composition, concentration, and in the amount of target(s), that is, $S_1 = S_2 = S_3$, etc. (page 8, lines 6-16). Furthermore, it is known to those skilled in the art that "microarrays," in general, are arrays of dots having a diameter from about 1 to 500 microns (page 9, lines 11-12). By using a microarray format, the methods of the present invention permit a substantial decrease in an amount of a sample required for testing as compared to conventional microtiter plate methods. For example, in one embodiment described in the Example 1 on page 18, only 1 microliter of a sample is required to produce as many as 1000 sample dots locations of a microarray, while a conventional 1536 microtiter plate would require the use of about 2 milliliters per plate. It is unexpected discovery of the present invention that a sample distributed into microdots of a microarray, provide sufficient quantities of a sample to be successfully tested with a probe or multiple probes specific for target biopolymer(s) contained in the sample.

Consistent with the above-discussed features of the present invention, all three independent claims, 1, 30, and 53 require the step (a) of "preparing a microarray of said sample by dispensing aliquots of said sample at discrete sites onto a substrate ..., wherein each of said aliquots contains the same amount of said target biopolymer [the term 'biopolymer' is substituted with the term 'nucleic acid' in claim 30 and the term 'analytes' in claim 53]." Thus, claims 1, 30, and 53 require forming a microarray by dispensing aliquots of a single sample containing the same amount of target biopolymer.

Jahn does not anticipate the instant independent claims 1 and 53 because he does not teach forming a microarray by dispensing aliquots of a single sample. Instead he teaches an immunoassay method that provides a parallel handling of

relatively large quantities (20μl samples) of a large number of different samples (abstract). Jahn teaches that "[a] grid of squares (1.8x1.8 cm) was drawn on nitrocellulose membrane filters... Each sample was adjusted to a spotting volume of 20 μl and spotted in three 6- to 7-μl portions on the center of a square, using a hair dryer for drying between the applications. The diameter of the spots was 1.2-1.5 cm" (p. 1684, under "Standard Procedure for the "Dot-Immunobinding assay.") Accordingly, in Jahn, each sample in its entirety is deposited in a single location on the filter. Additionally, since the printed spots in Jahn have a diameter of 1.2-1.5 cm, Jahn does not teach forming microarrays. Finally, Jahn has no teaching of each dot having the same amount of the analyte. To the contrary, different dots of John contain different samples with different quantities of analyte.

Jahn does not suggest the present invention. Jahn discloses a conventional dot-immunobinding assay of proteins in which a large number of different samples is spotted on nitrocellulose membrane filters, incubated sequentially with specific antibodies and ^{125}I-labeled protein A, and assayed for radioactivity (abstract). Unlike the present invention, Jahn does not utilize a microarray format for his assay. Instead, as discussed above, Jahn prints large dots with a diameter of 1.2-1.5 cm and hybridizes them with probes. Based on the teachings of Jahn, those skilled in the art would not have recognized that a microarray analysis of a single sample might be successfully performed, where the array is formed by subdividing the single unknown sample into a microarray of numerous aliquots having an equivalent amount of a target biopolymer and having a size of from 3 to 5 orders of magnitude less than those of Jahn. Thus, a method step of "preparing a microarray of said sample by dispensing aliquots of said sample at discrete sites onto a substrate," "wherein each of said aliquots contains the same amount of said target biopolymer" is not obvious in view of Jahn. Therefore, independent claims 1 and 53 are patentable over Jahn. Claims 3, 7, 9, 11-14, 17-18, 22-28, 55-58, 62, and 66-71 depend from claims 1 and 53, directly or indirectly, and, thus, they are patentable over Jahn for at least the same reasons as claims 1 and 53.

Claims 1, 7, 8, 10, 13-14, 17-18, 21-24, 26, 28, 30, 37-40, 44-47, 49, 53, 55-57, 62, 66-68, and 70 are rejected under 35 U.S.C. § 102(b) as being anticipated by Shuber et al., *Human Molecular Genetics* (1997), Vol. 6(3), pages 337-347 ("Schuber"). This rejection is respectfully traversed.

Shuber does not anticipate the instant independent claims 1 and 53 because he does not teach forming a microarray by dispensing aliquots of a single sample. Instead, similarly to Jahn, Schuber teaches a diagnostic assay for analyzing large numbers of different samples (>500) simultaneously for a large number of known mutations (abstract). Shuber teaches blotting amplified DNA samples, each positive for one of 106 mutations, on a membrane (legend to Figure 3). Shuber utilizes a conventional 96-well dot-blot apparatus and applies 30 μl of sample solution to each well (page 345, under "Dot-Blots"). Accordingly, in Shuber "[e]ach dot represents the multiplex amplification performed on one patient DNA for one disease gene only" (legend to Figure 1). The typical size of dots obtained with a 96-well apparatus of the type used by Shuber is a few millimiters in diameter (see, for example, attached specification for a similar device). Thus, in Shuber, each sample is deposited in a single location on the membrane. Additionally, since the printed spots in Shuber have a diameter of a few millimeters, Shuber does not teach forming microarrays. Finally, Shuber has no teaching of each dot having the same amount of the analyte. To the contrry, different dots of Shuber contain different samples with different quantities of analyte.

Shuber does not suggest the present invention. Shuber discloses a conventional, multiplexed allele-specific diagnostic assay for analyzing large numbers of different samples (title and abstract). Unlike the present invention, Shuber does not utilize a microarray format for his assay. Instead, as discussed above, Shuber prints large dots with a diameter of a few millimiters and hybridizes them with probes. Based on the teachings of Shuber, those skilled in the art would not have recognized that a microarray analysis of a single sample might be

successfully performed, where the array is formed by subdividing the single unknown sample into a microarray of numerous aliquots having an equivalent amount of a target biopolymer and having a size of several orders of magnitude less than those of Jahn. Thus, a method step of "preparing a microarray of said sample by dispensing aliquots of said sample at discrete sites onto a substrate," "wherein each of said aliquots contains the same amount of said target biopolymer" is not obvious in view of Shuber. Therefore, independent claims 1, 30, and 53 are patentable over Shuber. Claims 7, 8, 10, 13-14, 17-18, 21-24, 26, 28, 37-40, 44-47, 49, 55-57, 62, 66-68, and 70 depend from claims 1, 30, and 53, directly or indirectly, and, thus, they are patentable over Shuber for at least the same reasons as claims 1, 30, and 53.

Claims 1-19, 21-42, 44-64, and 66-72 are rejected under 35 U.S.C. § 103(a) over Shuber in view of Balch et al., U.S. Patent 6,312,960 B1 (the '960 patent), November 6, 2001. This rejection is respectfully traversed.

As discussed above, independent claims 1, 30, and 53 are patentable over Shuber. The '960 patent does not remedy the defects of Shuber and is not relied upon by the Examiner for such. The Examiner cites the '960 patent for teaching certain features of conventional probe arrays and methods of their making. However, the '960 patent has no teaching whatsoever of methods of making sample arrays, much less methods of preparing a microarray of a single sample by dispensing aliquots of the sample at discrete sites onto a substrate (column 4, lines 21-41).

In the probe microarrays, high concentrations of homogeneous probe solutions are applied to the substrate and reacted with large quantities of sample containing the target. On the other hand, in the present invention, unexpectedly, relatively small quantities of a heterogeneous sample containing a number of biomolecules, in addition to the target, are immobilized on the substrate and successfully reacted with a solution containing one or several probes. As discussed

on page 7, lines 19-25, the present invention provides substantially higher hybridization efficiency relative to that of reverse blot oligonucleotide probe microarray assays, since according to the present invention, the target is randomly attached to the substrate and, thus, more accessible to the probe. In contrast, in the conventional reverse blot format, the probe is attached at a high molecular surface density which does not allow for the efficient binding of the target to the probe due to steric hindrance.

Therefore, a teaching of probe microarrays by the '960 patent does not overcome the deficiencies of Shuber. Thus, independent claims 1, 30, and 53 and their dependent claims 2-19, 21-29, 31-42, 44-52, 54-64, and 66-72 are patentable over Shuber in view of the '960 patent.

Claims 1-72 are rejected under 35 U.S.C. § 103(a) over Shuber in view of the '960 patent, further in view of Sirvio et al., U.S. Patent No. 5,532,311 (the '311 patent). This rejection is moot with respect to claim 33 due to the cancellation of the claim. With respect to claims 1-32 and 34-72, this rejection is respectfully traversed.

As discussed above, independent claims 1, 30, and 53 are patentable over Shuber in view of the '960 patent. The '960 patent does not remedy the defects of Shuber and the '960 patent, and is not relied upon by the Examiner for such. The Examiner cites the '311 patent for teaching a substrate being wetted with an organic modifier selected from dextran sulfate or polyacrylic acid. The '311 patent provides a general teaching of processes for modifying surfaces. However, the '311 patent has no teaching whatsoever of methods of making microarrays, much less of methods of preparing sample microarrays. Therefore, independent claims 1, 30, and 53 and their dependent claims 2-29, 31-32, 34-52, and 54-72 are patentable over Shuber in view of the '960 patent and the '311 patent.

In view of the foregoing, it is respectfully submitted that the application is in condition for allowance. Reexamination and reconsideration of the application, as amended, are requested.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles, California telephone number (213) 337-6700 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-1314.

Respectfully submitted,
HOGAN & HARTSON L.L.P.

Date: April ___, 2003

By: _____
Wei-Ning Yang
Registration No. 38,690
Attorney for Applicant(s)

500 South Grand Avenue
Suite 1900
Los Angeles, California 90071
Phone: 213-337-6700
Fax: 213-337-6701

ATTACHMENT

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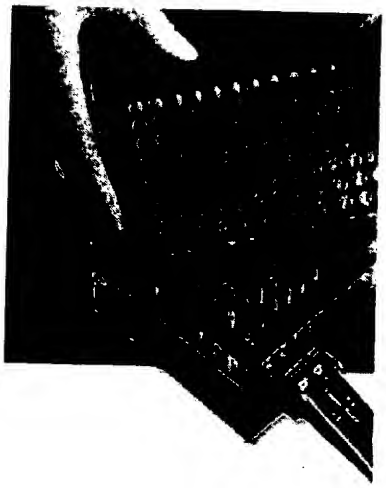
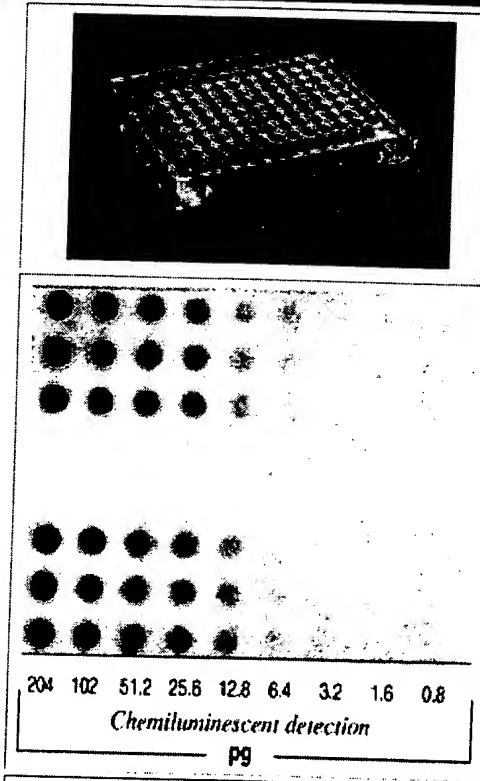
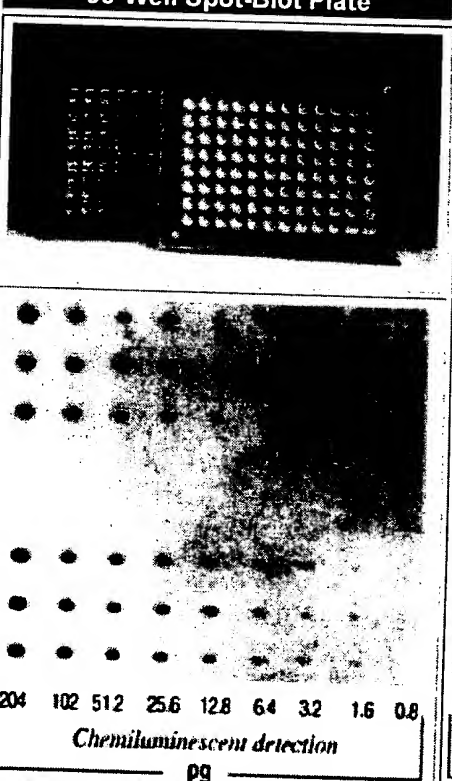
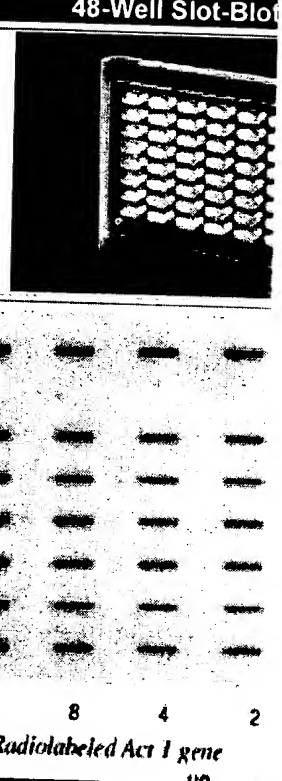


The Minifold I system consists of four basic components: sample well plate, filter support plate, vacuum plenur clamping plate (see diagram). The sample well plate is available in three configurations for producing spots, d below). All three plates are interchangeable and can be purchased as an accessory plate in conjunction with a system. The tension on the clamping is adjustable, permitting use of a variety of blotting and filtration media.

96-Well Dot-Blot Plate	96-Well Spot-Blot Plate	48-Well Slot-Blot
		
204 102 51.2 25.6 12.8 6.4 3.2 1.6 0.8 *Chemiluminescent detection* pg	204 102 51.2 25.6 12.8 6.4 3.2 1.6 0.8 *Chemiluminescent detection* pg	16 8 4 2 *Radiolabeled Act 1 gene* µg
Unique O-ring design - ensures discrete dot formation without leakage of samples by cross lateral flow. **Generates even, uniform dots** - the standard dot-blot system which eliminates uneven test areas resulting from manual sample application. **Two materials available** - standard	**Small volume required** - sample volumes as low as 25 µl can be applied using less of your precious sample. **Very high signal intensity** - 2 mm2 sample application area results in increased signal intensity. **Standard microtitration format** - 96	**Preferred format for densi scanning** - slots can be eas **Concentrated signal** - 6 m application area results in h intensity **Easy to survey format** - 4 your membrane are easier t samples.

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acrylic or autoclavable Delrin®.

Specifications -
Material: Acrylic or Delrin
Filter Area: 12.5 mm2/dot
Max Capacity: 500 µl/well, 96 wells
Pressure: 0.9 bar, vacuum

samples on a single membrane; same as the standard Minifold Dot-Blot system.

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Filter Area: 2 mm2/spot (1x2 mm)
Max Capacity: 200 µl/well, 96 wells
Pressure: 0.9 bar, vacuum

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Pressure: 0.9 bar, vacı

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Minifold I

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Minifold I Slot-Blot* System, complete (Acrylic)	1	10447940
Minifold I Dot-Blot * System, complete (Delrin™)	1	10447910
Minifold I Dot-Blot System and incubation plate	1	10484139
Replacement Parts		
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Minifold I Slot-Blot plate	1	10447945
Minifold I Dot-Blot plate	1	10447915
Minifold I Clamping plate	1	10447960
Filter Support plate	1	10447913
Vacuum connector	1	10447966
Vacuum plenum	1	10447968
Orings	50	10447917
Accessory Products		
Incubation plate	1	10447919
Cutting template	1	10447901
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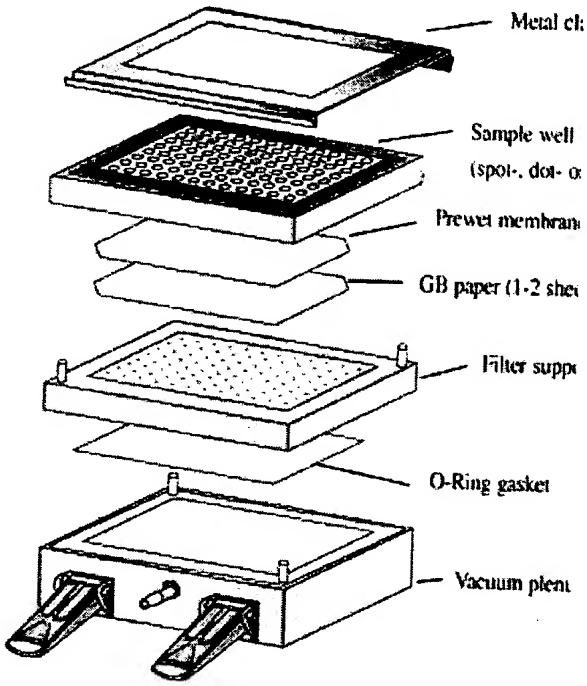
Nytran N, 0.45 µm	10	10416188
Optitran®, 0.2 µm	10	10439388
Optitran, 0.45 µm	10	10439188
Protran®, 0.2 µm	10	10402488

MINIFOLD I EXPLODED DIAGRAM



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*Complete systems include: manifold apparatus, 5 sheets of Protran NC, 5 sheets of precut GB002 paper.

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